reducing hydrogenases (Kovács et al. 2005; Maróti et al. – in press). Furthermore the bacterium possesses nitrogenase activity, and the atmospheric N_2 fixing is accompanied by H_2 evolution.

The *hup* locus consists of seven genes (*hupSLCDHIR*). Some of these genes are well characterized, *hupC* encodes a cytochrome b-type protein involved in electron transfer (Palágyi-Mészáros et al. 2009), while *hupD* codes for an endopeptidase which plays a role in the maturation of the large subunit (HupL). HupR is a regulatory protein, a part of a transcriptional regulatory system. However little is known about the transcriptional organization and regulation of *hup* gene, function of *hupH* and *hupI* gene products.

According to the literature, HupH protein is required for the translocation of the H_2ase stuctural protein to the membrane by bonding to the small subunit, while HupI is a rubredoxin-type protein plays a role in the electron transfer (Manyani et al. 2005).

In order to investigate the role of *hupH* and *hupI* genes in frame deletion mutants were created and the phenotypical effects of the mutations were analyzed by measuring *in vivo* and *in vitro* hydrogenase enzyme activity. Results showed that the absence neither of HupI nor HupH cause a significant decrease in Hup uptake activity.

The transcription of *hup* genes was investigated by reverse transcription coupled PCR, the results showed that *hupSLCDHIR* genes transcribe as a whole transcript.

Expression level of the *hup* genes was measured by quantitative real-time PCR in cells grown on various medium. Under nitrogen fixing conditions an enhanced *hup* mRNA level was observed, which indicates that the physiological function of Hup is somehow linked to the activity of nitrogenase enzyme complex. In standard non-nitrogen fixing growth conditions the *hupSL* transcription downregulated by both thiosulfate and succinate and upregulated by the inactivation of HupC. Therefore it was hypothesized that the redox status of the membrane/quinone pool controls the expression level of Hup hydrogenase.

To identify regulatory proteins which control the *hup* expression, mini Tn5 transposon based mutagenesis was carried out. A screening procedure was developed for identification of strains having Hup hydrogenase activity when the quinone pool is overreduced. *In vitro* hydrogenase uptake activity measurments were showed an appreciably increased Hup activity in the mutant and this points to the fact that the insertion of the transposon inactivated a gene which encodes a protein likely involved in the redox control of the expression of Hup hydrogenase.

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Clarifying the mechanism of T-cell apoptosis induced by cell-derived or low and high concentration of soluble recombinant galectin-1

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Galectin-1 (Gal-1) is a mammalian lectin with β-galactoside binding activity. It is expressed by numerous cell types and binds to cells and extracellular matrix components presenting glycoconjugates of N-acetyl-lactosamine. The most prominent biological function of Gal-1 is its anti-inflammatory effect which is predominantly exerted by induction of apoptosis of Th1 cells (1). Many studies have emerged analyzing Gal-1 signal transduction mechanism during T-cell apoptosis. However these data have resulted confusing knowledge due to using soluble recombinant protein although Gal-1 exerts its physiological function bound to the producing or neighboring cells or extracellular matrix components.

We have aimed to resolve this controversy by comparing cell death induced by low (1.8 μM, lowGal-1) and high (18 μM, highGal-1) concentration of soluble Gal-1. We show that lowGal-1 and highGal-1 trigger phosphatidylserine exposure, generation of rafts and mitochondrial membrane depolarization. In contrast, lowGal-1 but not highGal-1 are dependent on the presence of p56lck and ZAP70 and activates caspase cascade. The results allow the conclusion that the cell-death mechanism strictly depends on the concentration of Gal-1 (2).

Recombinant Gal-1 is always manipulated during purification and in apoptosis assays since it has to be in reduced form for functional conformation. To avoid this process we analyzed the role and mechanism of cell-derived Gal-1 in the apoptotic process. In co-culture system Gal-1 remains as a native, functional protein without any chemical modification and the apoptosis assay also avoids addition of reducing agent. We applied co-cultures of various cell lines producing Gal-1 as effectors and T-cells (activated peripheral blood cells or

Jurkat lymphoblasts) as targets. Both Jurkat and activated peripheral T-cells died when co-cultured with various Gal-1 expressing cells, but HeLa, a Gal-1 non-expressing cervix carcinoma cell line did not affect T-cell viability. Removing cell surface Gal-1 with lactose or knocking down Gal-1 expression in Gal-1 producing tumor cells resulted in the diminution of the cytotoxic effect of these cell lines. Moreover, transgenic expression of Gal-1 in HeLa cells or treating HeLa cells with recombinant Gal-1 (rGal-1) converted these cells cytotoxic. T-cell apoptosis required intimate interaction between the effector tumor and target T-cells since neither conditioned supernatant harvested from the tumor cells, nor physical separation of tumor and T-cells in the same medium triggered T-cell death. Mechanism of apoptosis by cell-bound Gal-1 was comparable to that of low concentration of soluble recombinant Gal-1. Requirement for p56lck and ZAP70 has been proved and both the decrease of mitochondrial membrane potential and caspase activation was detected in T-cell apoptosis triggered by tumor cell-derived Gal-1 (3).

Our results show that cell-derived Gal-1 and low concentration of the soluble lectin triggers identical pathway of T-cell apoptosis in contrast to high concentration soluble Gal-1 which act on a different fashion.

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Evolutionary and functional analysis of *Medicago truncatula* symbiotic genes on nodulating and non-nodulating plant species

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The availability of soil nutrients for plants is a major limiting factor regarding growth and productivity at many agronomically important areas. For this demand an evolutionary solution is the existence of symbiotic associations between plants and soil microbes that provide valuable macronutrients (e.g. P or N) for photosynthates as an exchange material from the plant. An ancient type of coexistence is the symbiosis with arbuscular mycorrhiza fungi. It is originated back to the appearance of the first land plants and present in the majority of land plant families. However, another, more recent type of symbiosis exists between nitrogen fixing soil bacteria and a narrow range of plants consisting of phylogenetically closely related species. During this symbiosis a new organ, the root nodule is formed that is specific for nitrogen fixation. Research on genes involved in the formation of these types of symbiotic associations showed that the two systems share genes supporting the idea that already existing elements of the more ancient program were recruited during the evolution of root nodule symbioses.

The mutual recognition of partners is a key process during the establishment of the symbiotic association. Specific molecules have been identified on both sides that are essential for triggering the symbiotic process, e.g. flavonoids secreted by the plants and the so-called Nod factors produced by the symbiotic bacteria. Due to the intensive ongoing research on symbiotic nitrogen fixation there is a rapid increase in the number of identified plant genes involved in these signaling events. LysM type receptor kinases (*MtLYK3*, *MtNFP*) together with the LRR receptor like kinase *MtDMI2* are needed to promote the most characteristic phenomenon of the early symbiotic signaling process: the perinuclear calcium level oscillation via nuclear pore complex elements (*LjNUP85*, *LjNUP133*) and a putative potassium ion channel *LjCASTOR* and *LjPOLLUX*/ *MtDMI1*. The signature of this so called calcium spiking is decoded and forwarded by a calcium-calmodulin dependent protein kinase (*MtDMI3*) via its phosphorylation substrate (*MtIPD3*) towards transcription factors (*MtNSP1*, *MtNSP2*, *MtERN*, *MtNIN*). Moreover, *NIN* and a cytokinin receptor needed for symbiotic nitrogen fixation (*MtCRE1*) are elements of the pathway that allows crosstalk between Nod factors of the symbiotic bacteria and plant cytokinins during nodulation. Living the days of the genomic era more and more `whole genome sequences' are accessible in the databases including plant genomes as well. Searching these databases makes possible to identify genes homologous to known symbiotic genes with high significance not only from legume genomes but also from non-nodulating plants. However, there are only a few papers published so far on evolutionary relationships of particular symbiotic genes and their homologues in different plant species (most recently Chen et al. 2009 on *LjCASTOR*/ *LjPOLLUX*/ *MtDMI1*). We have done systematic searches using the protein sequences of *M. truncatula* symbiotic genes as query and could identify new homologous genes from non nodulating plants highlighting some interesting aspects of their evolutionarily recruited functions. We have selected a few homologous proteins to explore for their possible function, with special regard on their possible ability of fulfilling symbiotic function as well.

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